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HLA-DR4–Associated T and B Cell Responses to Specific Determinants on the IA-2 Autoantigen in Type 1 Diabetes

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Autoantibodies to IA-2 in type 1 diabetes are associated with HLA-DR4, suggesting influences of HLA-DR4–restricted T cells on IA-2–specific B cell responses. The aim of this study was to investigate possible T–B cell collaboration by determining whether autoantibodies to IA-2 epitopes are associated with T cell responses to IA-2 peptides presented by DR4. T cells secreting the cytokines IFN-γ and IL-10 in response to seven peptides known to elicit T cell responses in type 1 diabetes were quantified by cytokine ELISPOT in HLA-typed patients characterized for Abs to IA-2 epitopes. T cell responses were detected to all peptides tested, but only IL-10 responses to 841–860 and 853–872 peptides were associated with DR4. Phenotyping by RT-PCR of FACs-sorted CD45ROhi T cells secreting IL-10 in response to these two peptides indicated that these expressed GATA-3 or T-bet, but not FOXP3, consistent with these being Th2 or Th1 memory T cells rather than of regulatory phenotype. T cell responses to the same two peptides were also associated with specific Abs: those to 841–860 peptide with Abs to juxtamembrane epitopes, which appear early in prediabetes, and those to peptide 853–872 with Abs to an epitope located in the 831–862 central region of the IA-2 tyrosine phosphatase domain. Abs to juxtamembrane and central region constructs were both DR4 associated. This study identifies a region of focus for B and T cell responses to IA-2 in HLA-DR4 diabetic patients that may explain HLA associations of IA-2 autoantibodies, and this region may provide a target for future immune intervention to prevent disease.

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Materials and Methods

Study subjects

Patients (n = 127) of up to 30 y of age were recruited within 6 mo of diagnosis of type 1 diabetes from clinics in West Yorkshire, Durham, and King’s College Hospital, London, U.K., and provided blood samples for analysis of autoantibody responses and for HLA genotyping with informed consent and approval from appropriate Ethics Committees (reference 08/
H1313/70). A subgroup of 58 of these patients aged between 12 and 30 y provided sufficient volumes of heparinized blood (>20 ml) delivered within 24 h of sample collection to laboratories at King’s College London for analyses of T cell responses to IA-2 peptides by cytokine ELISPOT. The characteristics of the subjects studied for autoantibody associations with HLA gene expression and T cell responses are summarized in Table I. There were no significant differences in characteristics between these two study groups. Five additional patients (three male, mean age 22 y) were recruited for phenotypic analysis of peptide responsive T cells.

DNA extraction and HLA genotyping

Study subjects were genotyped for class II HLA-DRB1 and DQB1 loci. Genomic DNA was extracted from cell pellets obtained after density gradient centrifugation of heparinized whole blood by proteinase K digestion followed by salt-ethanol precipitation. DNA was further purified using the Genomic DNA Clean and Concentrator kit (Zymo Research, Irvine, CA). Genotyping was performed by PCR amplification of genomic DNA, using sequence-specific primers (21, 22).

Ab analysis

Autoantibodies to radiolabeled GAD65, IA-2, and ZnT8 constructs were analyzed by radioligand binding assays, as previously described (23, 24). Abs to GAD65 were analyzed using constructs representing the full-length coding region of the human protein, whereas ZnT8 Abs were determined using constructs representing the C terminus of the molecule (aa 268–369) with both Thr252 and Arg255 variants. IA-2 constructs used were the cytoplasmic domain of IA-2 (IA-2c; residues 605–979) and truncated constructs (25) representing the JM domain (residues 605–693), PTP domain (residues 643–979), and the central region of the cytoplasmic domain (residues 643–937).

IA-2, GAD, and ZnT8 cDNAs were transcribed and translated in vitro in the presence of [35S]methionine using the TNT-Quick-Coupled Transcription/Translation System (Promega, Southampton, U.K.). Incorporation of label was determined by precipitation of the translated protein with 10% TCA, followed by scintillation counting. Radiolabeled protein (20,000 cpm in 20 μl) was incubated with 5 μl test sera for 16 h at 4°C in wash buffer (10 mM HEPES [pH 7.4], 150 mM NaCl, 0.1% Triton X-100). Immunocomplexes were immunoprecipitated with Protein A-Sepharose and washed five times under vacuum filtration with wash buffer, followed by two washes in water. The quantity of immunoprecipitated radiolabeled Ag was determined by liquid scintillation counting (Plate Chomeloun V; LabLogic, Sheffield, U.K.). Ab levels (as World Health Organization labeled Ag was determined by liquid scintillation counting (Plate Chomeloun V; LabLogic, Sheffield, U.K.)).

Isolation of IL-10–secreting CD4+ T cells by FACS

To obtain preparations of T cells enriched in those responding to IA-2 peptides suitable for further phenotyping, PBMCs were seeded at a density of 2 × 10^6/ml into 5-ml round-bottomed polystyrene tubes in medium alone or with 10 μg/ml IA-2 peptide (841–860 or 853–872) and incubated for 18 h at 37°C in a 5% CO2 atmosphere. Where there were sufficient cells, PBMCs were also incubated with 20 μl/ml Cytostim (Milenyi Biotec, Bisley, U.K.) as a non–antigen-specific stimulus. Cells were recovered and IL-10–secreting cells labeled using an IA-2 peptide assay detection kit (Milenyi Biotec) according to the manufacturer’s instructions. Cells were costained with CD4 and CD45RO Ab (Milenyi Biotec) and a live/dead fixable stain (BioLegend, San Diego, CA). Sorting of live CD4+CD45RO+IL-10–secreting cells was performed on an FACS Aria (BD Biosciences, Oxford, U.K.) and data analyzed using FCS Express (De Novo Software, Los Angeles, CA).

Phenotyping of IL-10–secreting T cells by PCR

Total RNA was isolated from CD4+ CD45RO+ IL-10–secreting T cells immediately postsort using a PureLink RNA Micro Scale Kit (Life Technologies, Carlsbad, CA) and cDNA generated using Sensiscript Reverse Transcriptase (Qiagen, Hilden, Germany). PCR was performed with GoTaQ G2 (Promega) for 40 cycles with annealing temperature of 60°C using the following primer pairs: T-bet forward, 5′-GAT GTT GGG GTA CGT GGT CTT G-3′, T-bet reverse, 5′-CTT TCA ACA CTG CAC CCT TTT C-3′; GATA-3 forward, 5′-ACC GGC TTC GGA TGC AA-3′; GATA-3 reverse, 5′-TGC TCT CCT GGC TGC AGA C-3′ (30); retinoic acid–related orphan receptor γt (RORγt) forward, 5′-TTT TCC GAG GAT GAG AGT GC-3′, RORγt reverse, 5′-CTT TCA ACA TGG TCG CTA CA-3′ (31); FOXP3 forward, 5′-CAG CAC ATT CCC AGA GGT CCT C-3′, FOXP3 reverse, 5′-GGC TGT GAA GCA GTG GAT CAC CCA-3′; GATA-3 forward, 5′-GGC TGT GAA GCA GTG GAT CAC CCA-3′, GATA-3 reverse, 5′-TG CTC GCC AGT CCA TCA C-3′ (30). Control RT-PCR reactions were performed with RNA from total PBMCs to confirm appropriate functioning of all primer pairs. PCR products were run on a 3% agarose gel stained with ethidium bromide and visualized using a Gene Genius Bio Imaging System (Syngene, Cambridge, U.K.).

Statistical analysis

The analysis of T cell responses to autoantigens by cytokine ELISPOT presents a challenge because of the very low frequency of responding T cells in the samples, which introduces a high degree of uncertainty in assigning positive and negative responses in T cell assays. The frequent absence of cytokine-secreting cells with medium alone make the conventional approach to analysis of T cell responses by calculation of stimulation index impossible without further data manipulation, and cutoff values for positive responses were established for assays detecting cell proliferation after several days of Ag stimulation may be inappropriate for acute responses detected by ELISPOT. Our T cell response data contained a high proportion of zero values (see Results) and a skewed distribution of numbers of cells responding to IA-2 peptides. Because confidence in assigning positive values to T cell ELISPOT data increases with number of spot forming cells detected, the analysis needs to take into account differences in both the frequency of detectable responses and the number of spots within the responses detected. Lachenerbruch’s two-part statistic (33), which has been applied previously to immune response data with similar characteristics (34), was used to analyze T cell responses between different patient
Results

HLA association with IA-2 Ab epitopes

IA-2 Abs have been shown previously to be associated with the expression of HLA-DR4 (18). To further explore the role of this allele in regulation of IA-2 autoantibodies, we investigated HLA associations with Abs to constructs representing distinct epitopes in the JM and PTP domains of IA-2. In common with previous studies (18), a weak association of HLA-DR4 with Abs to the cytoplasmic domain of IA-2 was observed in 127 recent-onset type 1 diabetic patients \( (p = 0.03, \) Fisher exact test, Fig. 1A, Table I). Truncated constructs were used to assess the contribution of individual epitopes to the HLA-DR4 association observed. Abs to the IA-2605–693 construct, which contains three overlapping linear epitopes in the 611–640 region of IA-2, were more strongly associated with HLA-DR4 \( (p = 0.008; \) Fig. 1A). Likewise, Abs to the IA-2643–937 construct, representing a dominant conformational epitope within the PTP domain (831–860) of IA-2 (23), also showed a strong DR4 association \( (p = 0.003; \) Fig. 1A). In contrast, Abs to other epitopes in the PTP domain, as detected by reactivity to the IA-2643–979 construct, showed no significant HLA-DR4 association. To determine whether the HLA-DR4 association was secondary to a primary association at the linked diabetes-susceptibility HLA-DQ8 allele, patients were also typed for HLA-DQB1 alleles. The prevalence of IA-2 Ab specificities in HLA-DR4-DQ8 and HLA-DR4-DQ7 patients were similar, suggesting a primary association with HLA-DR4 (Fig. 1B). No associations of autoantibodies with other HLA-DRB1 or HLA-DQB1 alleles were detected.

T cell responses to IA-2 peptides

Responses to seven known IA-2 T cell determinants were analyzed in a subset of the patients by cytokine ELISPOT (Fig. 2). The magnitude of the responses varied considerably between subjects (range 1–62 spots/10⁶ PBMC), with the highest frequency of responding T cells observed for peptides in the region of 831–976 of the IA-2 molecule. There was no effect of sex on T cell responses, and the mean age at onset of responders and nonresponders to each peptide was similar in both the IL-10 and IFN-γ ELISPOT assays. IL-17 responses were too low for meaningful analysis (all responses <5 cells/10⁶ PBMCs).

HLA association of T cell responses

The association of the major diabetes-susceptibility HLA alleles with individual peptide responses was studied for IL-10 (Fig. 3A, 3B) and IFN-γ (Fig. 3C, 3D). The IL-10 response to peptide 841–860 was significantly associated with the expression of DR4 (Fig. 3A; \( p = 0.005, \) Lachenbruch’s two-part model) and also with the linked DQ8 allele (Fig. 3B; \( p = 0.015)\). However, no significant difference was observed in the frequency and magnitude of responses to this peptide between DR4-DQ8 and DR4-DQ7 subjects \( (p = 0.15)\), indicating that, like autoantibody responses, the primary HLA association is likely to be with DR4. IL-10 responses to peptide 853–872 were also significantly associated with DR4 (Fig. 3A; \( p = 0.046)\), but not to DQ8. No significant associations were detected for IFN-γ responses to IA-2 peptides with DR4, DQ8 (Fig. 3C, 3D), or DR3 (not shown). Neither IL-10 nor IFN-γ responses to PHA were associated with HLA alleles.

Subtyping of DR4-positive subjects indicated that although the majority (22 out of 34) expressed DRB1*0401, the DR4 subtypes DRB1*0402 (5 out of 34), 0404 (4 out of 34), 0405 (1 out of 34), 0408 (1 out of 34), and 0409 (2 out of 34) were also represented; one patient was heterozygous for DRB1*0405 and 0409. These subtypes differed from DRB1*0401 at 1–3 aa within the peptide binding site. The frequency and magnitude of IL-10 T cell responses to peptides 841–860 and 853–872 were not significantly different between DRB1*0401 subjects and those with other DR4 subtypes (peptide 841–860: frequency 53 versus 50%, magnitude 4.4 versus 1.7 responders/10⁶ PBMC; peptide 853–872: frequency 65 versus 40%, magnitude 4.4 versus 1.7 responders/10⁶ PBMC for 0401 and non-0401 patients, respectively). No additional associations of T cell responses with HLA were detected following DR4 subtyping.

Association of T cell responses with Abs

T cell responses to individual peptides were further analyzed to investigate associations with Abs to subdomains of IA-2 (Fig. 4). IL-10 responses to peptide 841–860 were significantly associated with Abs to the JM domain (Fig. 4A; \( p < 0.001, \) Lachenbruch’s two-part model) and, to a lesser extent, with Abs to the PTP domain (Fig. 4C; \( p = 0.037)\). IL-10 responses to peptide 853–872 were significantly associated with Abs to the central region (Fig. 4B; \( p = 0.032)\). No further Ab associations were observed with T cell responses to individual peptides for either IL-10 or IFN-γ.

Phenotyping of IL-10–secreting T cells

IL-10 is secreted by many T cell subsets, depending on the context of Ag stimulation, so additional phenotypic analysis of T cells secreting IL-10 in response to IA-2 peptide stimulation is required to understand the potential role of these cells in disease. Cells secreting IL-10 in response to peptides 841–860 and 853–872 were labeled using a cytokine secretion assay (Miltenyi Biotec), which captures the cytokine on the cell surface as it is released.

Table I. Characteristics of groups of type 1 diabetic patients analyzed for HLA associations with HLA gene expression and for T cell responses

<table>
<thead>
<tr>
<th>HLA-Ab Associations</th>
<th>T Cell Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>127</td>
</tr>
<tr>
<td>Age at diagnosis (mean ± SD)</td>
<td>17 ± 5.6</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>68.5</td>
</tr>
<tr>
<td>Autoantibodies (% positive)</td>
<td></td>
</tr>
<tr>
<td>IA-2A</td>
<td>68.5</td>
</tr>
<tr>
<td>GADA</td>
<td>79.5</td>
</tr>
<tr>
<td>ZnT8A</td>
<td>59.0</td>
</tr>
<tr>
<td>HLA (%)</td>
<td></td>
</tr>
<tr>
<td>DR3/4</td>
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</tr>
<tr>
<td>DR4/x</td>
<td>33.9</td>
</tr>
<tr>
<td>DR3/x</td>
<td>26.0</td>
</tr>
<tr>
<td>Non-DR3/4</td>
<td>12.6</td>
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Flow cytometric analysis of peptide-stimulated cells detected low numbers of IL-10–responding cells above background in IA-2 Ab-positive patients, consistent with the data obtained by ELISPOT assay (Fig. 5A, 5B). Live CD4+CD45RO+ T cells from three patients were subsequently flow sorted and analyzed for expression of transcription factors associated with differentiation of distinct T cell subsets by RT-PCR. T cells responding to 841–860 and 853–872 peptides were found to express T-bet or GATA-3 (Fig. 5C), transcription factors associated with Th1 or Th2 lineage, but not FOXP3 (regulatory T cell) or Rorγt (Th17). In contrast, T cells secreting IL-10 in response to stimulation with CytoStim, which activates T cells by cross linking of TCR, showed predominantly FOXP3 expression, consistent with regulatory T cells being dominant within the general IL-10–responsive memory T cell population.

Discussion

The observation that appearance of autoantibodies in early life is associated with expression of high-diabetes-risk HLA alleles (35) and the associations of autoantibodies to insulin, GAD, and IA-2 with specific alleles in the class II region of the HLA in new-onset type 1 diabetes (18, 19, 36, 37) support the view that HLA genes mediate their effects on disease susceptibility by influencing immune responses to islet autoantigens. Because HLA associations of individual autoantibody specificities do not precisely mimic HLA associations with disease, it has been argued that immune responses detected by autoantibody assays are unlikely to be directly involved in disease pathogenesis (19). However, the HLA region contains several loci conferring susceptibility to type 1 diabetes, with independent associations detected at HLA-DQ, HLA-DR, HLA-B, and HLA-A loci (38). This may reflect multiple effects of HLA genes on autoimmune responses to different islet autoantigens with the overall genetic susceptibility representing the combination of these effects. The progressive nature of appearance of Abs to islet autoantigens (12, 39) and the fact that highest risk for progression to type 1 diabetes is conferred by the presence of autoantibodies to multiple autoantigens rather than any single Ab specificity (15, 40) is consistent with spreading of responses to different immune targets being a crucial element of disease progression, and each of these responses is likely to have a different requirement for optimal Ag presentation on HLA molecules. Knowledge of how each of the products of diabetes-associated HLA genes regulates T and B cell responses to specific determinants on autoantigens is required to fully understand the molecular basis of HLA-mediated susceptibility to type 1 diabetes. The results of the current study provide, for the first time to

![FIGURE 1. Association of IA-2 Abs with HLA. Subjects grouped according to the expression of DR4 (black bars) or non-DR4 (open bars) HLA alleles (A) or DR4-DQ8 (black bars) or DR4-DQ7 (open bars) haplotypes (B) were analyzed for Ab reactivity to the IA-2ic, JM, central region (CR), or PTP domain constructs. Data are presented as percentage positivity for each construct. n = 127. The significance of differences between frequencies of Ab-positive patients in DR4 and non-DR4 groups are indicated: *p < 0.05, **p < 0.01.

![FIGURE 2. T cell responses to IA-2 peptides. The number of responding cells per million PBMCs detected in ELISPOT assays and representing cells responding to each IA-2 peptide tested are presented as scatter plots for IL-10 (A) and IFN-γ (B). The numbers of patients tested for each peptide are indicated above the relevant scatter plot, and the number of zero values for each peptide below the plot.](http://www.jimmunol.org/)
our knowledge, evidence of HLA-DR4 associations with T cell responses to individual peptides derived from autoantigen and demonstrate a three-way relationship among HLA gene expression, T cell responses to specific IA-2 peptides, and the presence of autoantibodies to IA-2 epitopes, which support a role for T–B collaboration in the disease.

Previous studies have demonstrated that autoantibodies to epitopes in the JM domain appearing in early prediabetes are found predominantly in individuals expressing HLA-DR4 (41). The results of the current study show that this HLA-DR4 association is still evident at the time of clinical onset, and the data also show a strong HLA-DR4 association to autoantibodies to another defined epitope within the central region (831–860) of the IA-2 PTP domain. Abs to the JM and central region epitopes were found at similar frequency in HLA-DR4 patients expressing either HLA-DQ7 or HLA-DQ8, indicating that the effects of the MHC class II region on the IA-2 Ab responses are conferred primarily by HLA-DR4 gene products, rather than those of linked DQ alleles. The presence of Abs to other epitopes in the PTP domain showed no significant HLA-DR4 association, indicating stronger effects of the HLA region on autoantibody responses to JM and central region epitopes than those to other regions of IA-2. Hence, the use of assays that discriminate autoantibody responses to individual epitopes on islet Ags may be crucial to understand the relationships of B cell, T cells, and HLA-genes in the autoimmune responses to islet autoantigens in type 1 diabetes.

T cell responses to individual IA-2 peptides were also found to be HLA-DR4 associated, a novel finding because previous demonstrations of HLA associations with T cell responses have required pooling of data from experiments with multiple peptides from different islet Ags (20). These associations were restricted to T cell responses to two overlapping peptides within the central region of the IA-2 PTP domain. The strongest association was found for peptide 841–860, originally identified as a T cell epitope in dia-
betes by defining the specificity of T cell lines derived from patients (27) and not previously shown to have links with HLA-DR4. The second peptide, 853–872, was identified as a naturally processed T cell epitope by elution of peptides from HLA-DR4 molecules isolated from IA-2–loaded B cells (20). T cell responses to several other peptides eluted from DR4 molecules in the latter study did not show such associations in our experiments. Although the majority of DR4 patients in our study were of DRB1*0401 subtype, a proportion (14 out of 34) expressed other DR4 subtypes (DRB1*0402, 0404, 0405, 0408, or 0409), which differ at 1–3 aa within the region of HLA-DR4 molecule representing the peptide binding site. The frequency and magnitude of T cell responses to the 841–860 and 853–872 peptides were not significantly different between patients with DR*0401 and other DR4 subtypes, suggesting that individual peptide presentation by various HLA-DR4 subtypes can tolerate small differences in the HLA-DR4 β-chain sequence. Much larger studies are required to determine the contribution of the individual DR4 subtypes to IA-2 peptide presentation. T cell responses to the 841–860 and 853–872 peptides showed associations with Abs to the JM domain and the central PTP domain epitope, respectively, which themselves are DR4 associated. Hence the study establishes direct links between HLA-DR4 expression, T cell responses to specific IA-2 determinants, and autoantibody responses to specific IA-2 epitopes, consistent with cooperation between T and B cells at the level of Ag presentation. Abs to specific epitopes may therefore be effective surrogate markers of underlying T cell responses and may prove valuable for monitoring of autoimmune responses or for selection of candidates for Ag-specific immune intervention.

Studies on the natural history of the B cell responses to islet Ags in early prediabetes have shown that the JM domain of IA-2 is an early autoantibody target, with Abs to epitopes in the JM domain found almost exclusively in individuals expressing HLA-DR4 (17, 41). In several individuals in whom early Ab responses are restricted to the JM domain, autoantibody reactivity subsequently spread to epitopes in the PTP domain of IA-2. Our finding that T cell responses to two overlapping determinants in the 841–872 region of the PTP domain of IA-2 are linked to Abs to the JM and PTP domains may be relevant to our understanding of this determinant spreading. T cell responses to the 841–860 peptide were associated with Abs to both JM and PTP domain epitopes. Although the JM and PTP determinants are separated by >200 residues in the primary structure of IA-2, these regions may be closely aligned in the three-dimensional structure, such that B cell recognition of JM domain epitopes may facilitate presentation of determinants within the PTP domain. Evidence for close structural relationships between the JM and central PTP domain epitopes comes from competitive binding studies of IA-2 Abs in which mouse mAbs directed to JM domain epitopes were shown to block IA-2 binding of the human monoclonal IA-2 autoantibody M13 (42), now known to bind epitopes in the 831–860 region of IA-2 (23).

In common with several other studies of T cell responses in type 1 diabetes (28, 43–45), T cells were detected responding to peptides derived from islet autoantigens by secretion of both proinflammatory cytokines (IL-10 and IFN-γ) and proliferation as measured by ELISPOT. T cells were analyzed for the presence of Abs to the JM domain, central PTP domain, or entire PTP domain of IA-2. T cell responses to IA-2 peptides were significantly associated with Abs to the JM domain (p < 0.01), the central region (CR) of the IA-2 PTP domain (p < 0.05), and the entire PTP domain (p < 0.001).
flammatory (IFN-γ) and anti-inflammatory (IL-10) cytokines. T cell responses with >15 responders/10^6 PBMCs were only detected to the 831–850, 841–860, 853–872, and 955–976 IA-2 peptides in IL-10 and IFN-γ assays. Although IFN-γ T cell responses were detected at similar frequency to those in the IL-10 assay, statistically significant associations of T cell reactivity with HLA-DR4 or IA-2 autoantibodies were only observed in the IL-10 assay. A major question is whether these IL-10 responses represent pathogenic or protective immune responses. Type 1 diabetes has been considered a Th1-mediated disease, with T cell responses to IA-2 and proinsulin peptides skewed toward production of IFN-γ (28). IL-10 clearly has anti-inflammatory properties and detection of T cells secreting IL-10 in response to cytokines from islet Ags has been interpreted as indicative of immune regulation (28). However, it is now recognized that IL-10 is not produced exclusively by Th2 or regulatory T cells but is also secreted, depending on the context of Ag exposure, by virtually all CD4+ helper subsets, potentially as a mechanism to self-regulate and prevent bystander damage (46). Modification of our cytokine secretion assay to allow capture and fluorescent labeling of IL-10 on the surface of responding T cells allowed purification of T cells responding to the 841–860 and 853–872 peptides for phenotypic analysis by flow cytometry and RT-PCR. The results demonstrated that the peptide-responsive CD4+ CD45RO^hi T cells express transcription factors associated with Th2 (GATA-3) or Th1 (T-bet) differentiation, whereas FOXP3, typical of regulatory phenotype, is not expressed. These results suggest that the conditions used for restimulation of memory T cells with peptide in vitro may not be sufficient for induction of cytokines normally associated with the parental phenotype. Similar results have been reported by the Zielinski group (47), in which Th17 memory cells that were found to produce IL-10 when restimulated in vitro in the absence of cytokines that promote the Th17 phenotype. It is clear that IL-10 secretion by T cells per se cannot be regarded as a reliable marker of immune regulation and that careful phenotyping of peripheral blood T cells producing IL-10 on islet Ag stimulation is essential to understand their role in the pathogenesis of type 1 diabetes. The detection of T cells responding to the 841–860 and 853–872 peptides with Th2 and Th1 phenotypic markers suggest that the responding cells may have roles both in supporting Ab production and in driving inflammatory responses.

Studies on T cell responses in human type 1 diabetes suggest a diversity of specificity (reviewed in Ref. 48) and phenotype that may indeed be expected following spreading of immune responses during a prolonged period of inflammation that may precede disease onset. Diabetes-specific autoreactive CD4+ T cells in peripheral blood are found within the CD45RO^hi memory population, but interpretation of T cell data can be complicated by the
detection of naïve T cell responses that are irrelevant to disease (49). T cell responses to the 841–860 and 853–872 peptides are of particular interest because of their association with a major disease susceptibility allele, their link to autoantibodies in the JM domain that feature early in the autoimmune response to IA-2, and their localization of within the PTP domain that suggest involvement of specific T cell determinants early in the autoimmune response to IA-2, and particular interest because of their association with a major disease susceptibility allele.


disclosures

The authors have no financial disclosures of interest.

References


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